Program/Abstract # 98
Cadherin-11 functions during mammary gland branching morphogenesis
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Several signal transduction pathways are active during mammary gland development. Parathryoid related hormone (PTHrP) is necessary for the maintenance and formation of the mammary gland. Canonical Wnt signaling is necessary for the formation of the mammary placode, and BMP4 has been shown to be involved in both the outgrowth the mammary bud, and nipple skin differentiation. Cadherin-11(Cdh11) is a mesenchymal cadherin that is expressed in mesenchymal cells of synovial joints, bone, and mammary gland. During mammary gland development, Cdh11 is expressed in the mammary mesenchyme. To further examine the role of Cdh11 has in the mammary gland, we used the Cdh11 knock-out (KO) mice and the mesenchymal cell line, C3H10T1/2 cells. Cdh11 knock-out mice showed a decrease in branching morphogenesis, thicker ducts, and enlarged terminal end buds at both day 1 and 5 week old mice ($n=5$, $p < .05$). Since this phenotype in the Cdh11 knock-out mouse appears to be similar to the over-expression of Wnt5a in the mouse mammary gland, several Wnts (Wnt1, Wnt4, Wnt10b, and Wnt5a) were examined for changes in expression. Wnt 1, 4, and 5a had increased RNA expression in the Cdh11 KO mouse. C3H10T1/2 cells were treated with PTHrP, LiCl or BMP4 to determine if they regulate Cdh11. PTHrP stimulated Cdh11 expression while LiCl, which mimics canonical Wnt signaling by inhibiting GSK, decreased Cdh11 expression. BMP4 did not affect Cdh11 protein expression. Therefore a complex regulation of Wnt and PTHrP signaling is required for mammary gland formation and Cdh11 is a focal point during this regulation.

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Program/Abstract # 99
NHE1: A Novel Determinant in Branching Morphogenesis
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Regulation of intracellular pH (pHi) is primarily a function of the ubiquitous plasma membrane Na+/H+ exchanger-1 (NHE1), which transfers cytosolic H+ across membranes in exchange for extracellular Na+. NHE1 protects cells against cytosolic acidification and its activation, which can occur via growth factor stimulation, has been shown to permit regulated cell adhesion, migration, and proliferation. Since the majority of these findings have been evaluated in immortalized cell lines, the function of NHE1 in regulating normal tissue morphogenesis has not been fully evaluated. Here we show that in a 4 day 3D tissue culture model of TGF-α-induced mammary branching morphogenesis, specific inhibition of NHE1 and the subsequent acidification of pHi with 10μM N-Methyl-N-isobutyl Amiloride (MIA) dramatically disrupts development such that structures resemble unpolarized large tissue masses. This phenotype is associated with unusual tissue protrusions and retractions (as determined by live video-microscopy), extensive proliferation, and prevalent ectopic expression of keratin-6. We had previously reported that noraml branching morphogenesis in our assay is dependent on TGF-α-induced ERK-1/2 activation. Here we report that NHE1 inhibition and subsequent acidification of pHi leads to more widespread and extensive activated ERK-1/2 three hours after TGF-α-stimulation. Moreover, inhibition of ERK-1/2 completely suppresses all MIA-associated phenotypes. These findings indicate that NHE1 regulation of pHi is essential for regulated ERK-1/2 signaling and ultimately normal tissue development.

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Program/Abstract # 100
Coordinate regulation of cell motility and intercellular adhesion during mammary branching morphogenesis
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Epithelial morphogenesis requires coordinate interactions between epithelial cells, stromal cells, soluble molecular signals and the extracellular matrix (ECM). Mammary branching morphogenesis further involves coordination of motility between luminal epithelial and myoepithelial cells. We have previously shown that normal mammary branching morphogenesis proceeds through a novel form of collective epithelial migration, without leading cellular extensions. We are now focused on identifying the molecular signals that initiate epithelial motility, that sustain ductal elongation and that spatially restrict migratory epithelial cells and prevent their dispersal into the ECM. We take a combined imaging and molecular genetic approach to dissect the tissue level process of branching morphogenesis into a series of discrete changes in the properties and behaviors of individual epithelial cells. We have identified critical roles for microtubule dynamics in the restraining myoepithelial cells population and Rac signaling in the elongating luminal epithelial cells. We are now focused on dissecting the role of E-cadherin signaling in regulating the migratory behavior of mammary epithelial cells in different ECM microenvironments. We have identified conserved and microenvironment-specific roles for calcium based intercellular adhesion more generally and for the E-cadherin pathway specifically. Our current work focuses on identifying the critical molecular signals in the ECM that determine cellular invasion strategies and that regulate tissue integrity during morphogenesis.

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Program/Abstract # 101
The coupling mechanism to generate synchronized oscillation of segmentation clock in mouse
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The periodicity of somites is established by a clock mechanism which regulates cyclic gene expressions in the presomitic mesoderm (PSM). In zebrafish, the gene oscillations in individual cells are synchronized with neighboring cells via so-called coupling mechanism utilizing Notch signaling. However the synchronizing mechanism in mouse somitogenesis is not clarified yet. To address this problem, I employed mosaic analyses using chimera embryos consist of two types of cells, wild-type and mutant lacking the Notch signaling component. In Dll1 chimera embryos, cyclic gene expressions were detected as abnormal broad patterns throughout PSM, indicating that Notch signaling through Dll1 is required for synchronized oscillation. Next, I focused on Lunatic fringe (Lfng) that is a core regulator of the clock in mouse. Lfng had been shown as a cell autonomous negative regulator of Notch activity. In the chimera embryos consist of wild-

type and Lfng-null cells, the pattern of cyclic genes was more severely affected compared with the Dll1chimera. In addition, I found that Lfng might work in non-cell autonomous manner, since Notch activity was found to be positive or negative in Lfng KO cells in the chimera embryo, although all cells show Notch activity in a simple Lfng KO embryo. These results suggest that Lfng is not only a key regulator of the clock but also plays an important role in the coupling mechanism.

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Program/Abstract # 102
Roles of Tbx2b during asymmetric brain development
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The molecular processes involved in establishing left-right (L-R) asymmetry of the vertebrate nervous system are not well understood. The zebrafish epithalamus is comprised of the bilateral habenular nuclei, left-sided parapineal, and medially located pineal. The parapineal is apparentat 28-31 hours post fertilization (hpf) as a group of cells migrating leftward from the anterior medial region of the pineal anlage, to lie adjacent to the left habenula. The parapineal is required for many asymmetries in anatomy and gene expression between the habenular nuclei. We have shown that T-box containing transcription factor 2b (Tbx2b) is required for parapineal formation. In tbx2b^{c144} mutants, parapineal cells are not correctly specified and fail to migrate. To investigate the roles of Tbx2b in parapineal migration, we are conducting high-resolution lineage labeling, and have found a left-of-midline bias for their origin. We are currently examining the fate of parapineal precursor cells in tbx2b^{c144}. We are also testing candidate regulators and targets of Tbx2b. In the retina, the transcription factor Nr2e3 is an antagonist of Tbx2b during photoreceptor specification. Our results suggest a similar antagonism between Nr2e3 and Tbx2b in parapineal development. Total cadherin activity is reported to be downregulated in Tbx2b morphants; our data suggests that N-cadherin is specifically required for parapineal cohesion. Future studies will involve tissue-specific tbx2b over-expression studies as well as high throughput transcriptome analysis to identify tbx2b transcriptional targets.

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Program/Abstract # 103
Cell shortening, basal constriction and epithelial relaxation, in the developing vertebrate brain, are regulated by non-muscle myosins
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Vertebrate brain morphogenesis includes a complex set of processes that result in correct folding of the neuroepithelium, so that it packs correctly into the skull, and correctly shapes the brain ventricles, a system of connected cavities containing cerebrospinal fluid. Multiple cell shape changes underlie these processes, including cell shortening and basal constriction, a previously undescribed morphogenetic mechanism. Myosins and their regulatory proteins comprise a huge and diverse gene family, which control these shape changes. We are analyzing two regions of the developing zebrafish brain in which we have shown that myosin regulation is pivotal. The first region is the midbrain-hindbrain boundary constriction (MHBC), which forms through cell shortening and basal constriction. At the MHBC both non-muscle myosin IIA and IIB have necessary and highly synergistic function. MHBC cells with decreased myosin II function neither shorten nor basally constrict. This is the first demonstration that myosin II function is required for cell shortening during brain development. The second region is the hindbrain, where, in contrast to the MHBC, inhibition of myosin II function is required for normal morphogenesis. In particular, inhibition of myosin activity by myosin phosphatase is required for normal cell shape and relaxation of the hindbrain epithelium to allow for ventricle expansion. These data indicate that regulation of myosin II, by creating a balance between myosin activation and inactivation, is critical for multiple processes during brain morphogenesis.

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Program/Abstract # 104
Vgl-2a is Required for Neural Crest Cell Survival During Zebrafish Craniofacial Development
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The development of the vertebrate cranial skeleton results from the specification, growth, patterning, and morphogenesis of tissues derived from all three germ layers in response to a complex network of reciprocal signaling. While many genes involved in these processes have been identified, others remain uncharacterized. We have identified a gene, vgl-2a, which is expressed in the pharyngeal endoderm and ectoderm surrounding the neural crest derived mesenchyme of the pharyngeal arches in zebrafish. We have found that reducing expression of vgl-2a in zebrafish embryos using Morpholino antisense oligonucleotides results in increased neural crest cells death, a defect in endodermal pouch morphogenesis, and subsequent reduction of cranial cartilages. We have also demonstrated that expression of vgl-2a within the arches is regulated by FGFs and retinoic acid, suggesting that vgl-2a may represent an intersection of these signaling pathways in pharyngeal arch development.

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Program/Abstract # 105
Requirements for fat4 and atr2a in shaping the zebrafish craniofacial skeleton
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Little is known about the mechanisms of cell-cell communication necessary to assemble skeletal elements of appropriate size and shape. Skeletalprogenitors may behave as coherent units by communicating via the planar cell polarity (PCP) pathway. In Drosophila, two sets of factors control PCP independently: the Fat and the Stan systems. While a requirement for components of the Stan system was recently demonstrated in regulating the oriented divisions of chondrocytes and cellular intercalation in long bones, a role for the Fat system in skeletal development has not been reported. We find that mutants in two zebrafish orthologs, fat4 and atr2a, have defects in the neural crest-derived craniofacial skeleton: mutant skeletal elements have irregular edges and chondrocytes fail to flatten or stack normally. Co-expression of fat4 and atr2a in the pharyngeal